SESSION 1

1 Effect of DNA Variants from BRCA2 Exons 19 and 20 on the Splicing Process by Hybrid Minigenes

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Study of the deleterious effect of genetic variants in disease genes is usually focused on the predicted effect on protein function. However, previous findings indicated that the disruption of the splicing process represent an important ethiopathogenic mechanism in Hereditary Breast Ovarian Cancer (HBOC). Nearly 200 different sequence variants of the BRCA genes have been identified in breast/ovarian cancer patients of the Prevention Programme of Castilla y León (Spain). A new mutation of the acceptor splice site of BRCA2 exon 20 (c.8488-1G>A) was detected in a breast cancer patient of the Genetic Counselling Unit of Castilla y León.

In order to investigate the effect of c.8488-1G>A on splicing and the splicing regulatory mechanisms, we performed bioinformatic analysis (NNSPLICE, ESEfinder and ESRsearch) together with splicing functional assays of this variant and all the mutations described in the BIC database of BRCA2 exons 19 and 20. Based on the creation/disruption of putative enhancers/silencers and disruption of the natural splice sites, we selected 16 of 77 BIC variants. We also created five novel variants (no detected in patients) that affected highly conserved Splicing Regulatory Elements (SREs). Moreover, by comparing human BRCA2 intron 19 (bp)with six different species, we found that human intron 19 contained an Alu sequence. In addition flanking regions to the Alu element were highly conserved. We therefore generated 2 deletions of the conserved sequences and a deletion of 272pb of the Alu sequence to test their roles in splicing.

Variant c.8488-1G>A was assayed by RT-PCR of patient lymphocyte mRNA and hybrid minigene experiments in HeLa cells. The rest of the variants were generated by direct mutagenesis of the wild type minigene of BRCA2 ivs18-exon19-ivs19-exon20-ivs20. Functional assays showed that 8 variants altered the splicing process by different mechanisms, indicating that any DNA change can disrupt it. Four variants disrupted the canonical splice sites (c.8488-1G>A; c.8488-2A>G; c.8487+1G>A; c.8487+3A>G) and were associated with total skipping of their exons; and two affected splicing enhancers/silencers (missense c.8378G>A and c.8609A>G+c.8611G>T) that caused partial splicing effects. Finally, it cannot be elucidated if missense c.8486A>T caused aberrant splicing due to the disruption either of the donor site of exon 19 or SREs (enhancer or silencer motifs). Neither of the deletions of the conserved intronic motifs had any splicing consequence. However, the elimination of the Alu sequence totally altered splicing of exons 19 and 20, suggesting a relevant role for constitutive splicing in humans.

In conclusion, aberrant splicing represent a relevant pathogenic mechanism in hereditary breast/ovarian cancer, and further study of these splicing defects may increase the proportion of families that benefit from genetic counselling. Splicing functional assays are valuable tools to discriminate between benign polymorphisms and pathogenic variants.